Gel Extraction of DNA (Spin Column Extraction)

NucleoSpin® PCR clean-up Gel Extraction Kit

1. **Excise gel slice containing DNA fragment of interest.**
   a. Gel electrophoresis fractionates DNA fragments.
   b. The gel is exposed to UV to find the DNA fragments (stained by Ethidium bromide).
   c. The goal DNA band is identified.
   d. Physically remove the slice of gel contains the goal DNA with clean surgical blade.
2. **DNA Purification**
   e. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml Eppendorf tube.
   f. Add an 200 µL Binding Buffer (NTI) per 100mg gel
   g. Incubate the mixture at 50°C for 5-10 min or until the gel has completely melted.
   h. Place a NucleoSpin® PCR clean-up Gel column in a provided 2 ml collection tube.
   i. Apply 700 µl of the DNA/agarose solution to the NucleoSpin® PCR clean-up Gel column, and centrifuge at 11, 000 x g for 30s at room temperature.
   j. Discard liquid and place the NucleoSpin® PCR clean-up Gel column back into the same collection tube. For volumes greater than 700 µl, load the column and centrifuge successively, 700 µl at a time. Each NucleoSpin® PCR clean-up Gel column has a total capacity of 25 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.
   k. Add 700 µl of Buffer NT3 into the NucleoSpin® PCR clean-up Gel columnCentrifuge at 11,000 x g for 30s at room temperature to wash the column.Discard the flow-through and re-use the collection tube.
   l. Repeat step k with another 700µl of Buffer NT3
   m. Discard liquid and centrifuge the empty NucleoSpin® PCR clean-up Gel column for 1 min at 11,000 x g to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the NucleoSpin® PCR clean-up Gel column.
   n. Place a NucleoSpin® PCR clean-up Gel column into a clean 1.5 ml eppendorf tube. Add 15-30µl (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix and incubate at room temperature for 1 minute. Centrifuge for 1 min at 11,000 x g to

elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.